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Nonsupplemented Luteal Phase Characteristics after the Administration of Recombinant Human Chorionic Gonadotropin, Recombinant Luteinizing Hormone, or Gonadotropin-Releasing Hormone (GnRH) Agonist to Induce Final Oocyte Maturation in *in Vitro* Fertilization Patients after Ovarian Stimulation with Recombinant Follicle-Stimulating Hormone and GnRH Antagonist Cotreatment

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Replacing GnRH agonist cotreatment for the prevention of a premature rise in LH during ovarian stimulation for *in vitro* fertilization (IVF) by the late follicular phase administration of GnRH antagonist may render supplementation of the luteal phase redundant, because of the known rapid recovery of pituitary function after antagonist cessation.

This randomized two-center study was performed to compare nonsupplemented luteal phase characteristics after three different strategies for inducing final oocyte maturation. Forty patients underwent ovarian stimulation using recombinant (r-)FSH (150 IU/d, fixed) combined with a GnRH antagonist (antide; 1 mg/d) during the late follicular phase. When at least one follicle above 18 mm was observed, patients were randomized to induce oocyte maturation by a single injection of either r-human (h)CG (250 μg) (n = 11), r-LH (1 mg) (n = 13), or GnRH agonist (triptorelin; 0.2 mg) (n = 15). Retrieved oocytes were fertilized by either IVF or intracytoplasmatic sperm injection, depending on sperm quality. Embryo transfer was performed 3–4 d after oocyte retrieval. No luteal support was provided. Serum concentrations of FSH, LH, estradiol (E_2), progesterone (P), and hCG were assessed at fixed intervals during the follicular and luteal phase.

The median duration of the luteal phase was 13, 10, and 9 d for the r-hCG, the r-LH, and the GnRH agonist group, respectively ($P = 0.005$). The median area under the curve per day (from 4 d post randomization until the onset of menses) for LH was 0.50, 2.34, and 1.07 for the r-hCG, the r-LH, and the GnRH agonist group, respectively ($P = 0.001$). The median area under the curve per day for P was 269 *vs.* 41 and 16 for the r-hCG, the r-LH, and the GnRH agonist group, respectively ($P < 0.001$). Low pregnancy rates (overall, 7.5%; range, 0–18% per started cycle) were observed in all groups.

In conclusion, the nonsupplemented luteal phase was insufficient in all three groups. In the patients receiving r-hCG, the luteal phase was less disturbed, compared with both other groups, presumably because of prolonged clearance of hCG from the circulation and the resulting extended support of the corpus luteum. Despite high P and E_2 concentrations during the early luteal phase in all three groups, luteolysis started prematurely, presumably because of excessive negative steroid feedback resulting in suppressed pituitary LH release. Hence, support of corpus luteum function remains mandatory after ovarian stimulation for IVF with GnRH antagonist cotreatment. (*J Clin Endocrinol Metab* 88: 4186–4192, 2003)

THE USE OF GnRH agonist cotreatment in ovarian stimulation protocols for *in vitro* fertilization (IVF) results in a short luteal phase and clearly reduced pregnancy rates (1), unless luteal phase support is provided (2). In a recent study, the luteal phase in IVF protocols using GnRH agonist without luteal support was characterized by very high progesterone (P) and estradiol (E_2) concentrations during the early luteal phase (3). Subsequently, premature luteolysis occurred during the midluteal phase. The corpus luteum seemed to be driven by the human (h)CG bolus injection used to induce final oocyte maturation during the late follicular phase, because the decrease in P concentrations was strongly correlated with the decrease in serum hCG levels (3). Endogenous LH levels remained low throughout the luteal phase.

The reason for abnormal luteal function after ovarian stimulation for IVF remains open for speculation. Possible mechanisms involved include: 1) continued down-regulation, at-

Abbreviations: AUC, Area under the curve; E_2, estradiol; h, human; ICSI, intracytoplasmatic sperm injection; IVF, *in vitro* fertilization; M II, metaphase II; P, progesterone; r-, recombinant; TVS, transvaginal ultrasound.

tributable to preceding follicular phase GnRH agonist coadministration, may retard pituitary recovery (4, 5); 2) the induction of multiple follicle development *per se* could either directly or indirectly influence the duration of the luteal phase (6, 7); 3) the removal of large quantities of granulosa cells at oocyte retrieval may diminish the most important source of P synthesis by the corpus luteum, thus disrupting the luteal phase (8, 9); 4) supraphysiological levels of steroids [related to the higher number of corpora lutea (10)] during the early luteal phase could directly inhibit LH release via negative feedback actions at the hypothalamic-pituitary axis (11).

The recent availability of GnRH antagonists for the prevention of a premature LH rise in IVF has enabled luteal phase characteristics after ovarian stimulation to be studied in the absence of a GnRH agonist. In contrast to GnRH agonist-induced pituitary desensitization [which suppresses gonadotropin release for at least 2–3 wk after cessation of the GnRH agonist (3, 5, 12)], gonadotropin levels recover within 24 h after stopping the GnRH antagonist (13–15). It has therefore been widely speculated that luteal phase supplementation may no longer be required in cycles where GnRH antagonist cotreatment is applied (16). Recent data in intrauterine insemination seem to support this contention (17). However, in IVF cycles in which ovarian stimulation was combined with a GnRH antagonist, the duration of the luteal phase was reduced and LH levels were extremely low (18–20). The use of a GnRH antagonist also allows the reassessment of the midcycle hCG bolus on corpus luteum function, because hCG can now be replaced by either endogenous or exogenous LH to induce final oocyte maturation. Several studies have demonstrated the feasibility of inducing an endogenous LH surge by administering a bolus dose of GnRH agonist, both in patients treated with ovarian stimulation alone (21, 22) and in patients cotreated with a GnRH antagonist (23). Moreover, the recent availability of recombinant (r-)LH enables an exogenous LH surge to be used to induce final oocyte maturation during stimulation (24). The current study was designed to reexamine luteal characteristics after ovarian hyperstimulation for IVF. The nonsupplemented luteal phase characteristics in patients cotreated with GnRH antagonists were studied in women randomized to three different approaches for the induction of final oocyte maturation: r-hCG, r-LH, or an endogenous LH surge induced by a GnRH agonist bolus.

Patients and Methods

Patients

This prospective randomized two-center trial was approved by the local ethics review committees of both participating centers, and a signed written informed consent was obtained from all patients. Inclusion criteria were: 1) regular indication for IVF or IVF/intracytoplasmatic sperm injection (ICSI); 2) no more than 38 yr of age; 3) regular menstrual cycles (cycle length between 24–35 d); 4) both ovaries present; 5) absence of uterine abnormalities that could impair embryo implantation or pregnancy evolution; 6) body mass index, 18–29 kg/m^2; 7) no history of poor ovarian response (less than three oocytes in a previous IVF cycle); and finally, 8) no history of moderate or severe ovarian stimulation syndrome.

Study protocol

After a negative pregnancy test (Clearview, hCG II; Unipath Ltd., Bedford, UK), ovarian stimulation was initiated on cycle d 2 or d 3 using a fixed daily dose of r-hFSH (Gonal-F, Serono; 150 IU sc). The GnRH antagonist (Antide, Serono; 1 mg daily sc) was initiated on the day that the largest follicle was at least 14 mm in diameter (25) and was continued up to and including the randomization day.

When at least one follicle was at least 18 mm, randomization was carried out, by sealed envelopes, to one of three approaches for triggering final oocyte maturation. For both centers, a separate stratified randomization list was generated by computer. The three arms of this study were: 1) r-hCG (Ovidrel, Serono), 250 μg sc (26, 27); 2) r-LH (Luveris, Serono), 1 mg sc (24); or 3) GnRH agonist (Decapeptyl; Ferring, Hoofddorp, The Netherlands), 0.2 mg sc. Oocyte retrieval was performed 35 h later. Insemination took place either by routine IVF or by ICSI. In case of fertilization, a maximum of two embryos were transferred after 2–5 d of culture, according to local procedures. No luteal support was provided. In the absence of menstrual bleeding, a urine or serum pregnancy test was performed 15–20 d post randomization.

The ovarian response was monitored with transvaginal ultrasound (TVS). TVS was performed at fixed days in the follicular phase, *i.e.* stimulation d 1, d 6, and on the day of randomization. The frequency of additional TVS depended on the diameter of the largest follicle. When the largest follicle was no more than 12 mm, the patient returned 2 d later; whereas when the largest follicle was more than 12 mm, she returned the next day. This approach enabled the initiation of the GnRH antagonist when the largest follicle was at least 14 mm.

Blood sampling was performed on stimulation d 1, on the day the GnRH antagonist was initiated, on the day of randomization, just before the administration of the randomized medication, on the day of the oocyte retrieval, and every other day thereafter.

Hormone assays

Blood samples were centrifuged, and serum was frozen and stored at −20 C. Serum was assayed for FSH, LH, E_2, P, and hCG in the same laboratory. From each patient, hormone assays were performed in the same run. All measurements were performed by immunofluorometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). Intra- and interassay variations were, respectively, less than 5% and less than 8% for FSH; less than 3% and less than 6% for LH; less than 6 and less than 16% for P; less than 8% and less than 11% for E_2, and less than 6% and less than 7% for hCG.

Study design and statistical analysis

In a previous study of IVF patients treated with ovarian stimulation combined with a GnRH agonist, hCG (10,000 IU) for induction of final oocyte maturation, and no luteal support, we observed a mean maximum P level in the luteal phase of 230 ± 95 (SD) nM (3). We hypothesized that in the absence of hCG, a difference in maximum P levels of 80 nM could be expected. To test the hypothesis that substitution of hCG by an exogenous or endogenous LH surge leads to a reduction in maximum P levels of 80 nM with 90% power at a P value of 0.05 (two-sided, assuming the overall SD = 95 nM), 30 patients were needed for each group, *i.e.* a total of 90 patients.

When 40 patients had been included, the study was canceled prematurely because of observed premature luteal phase bleeding and extremely low pregnancy rates. Preliminary observations highlighted a wide variation in the luteal phase length, both within and between the study groups (Fig. 1). Moreover, the differences in median maximum P levels between groups were much larger than expected (Fig. 1). Therefore, it was decided to analyze the data by comparing the area under the curve (AUC) per day, in addition to maximum P levels. The AUC/d was calculated from d 4 after randomization (to exclude the influence of the LH surge) until onset of menses. The sum of the daily levels measured was divided by the number of days until menses occurred. This results in estimated mean concentrations of the various parameters per day. This method of calculation results automatically in linear interpolation of missing values, unless missing values occur at endpoints of the interval. However, this did not occur: five patients had one missing assay day, and one patient had 3 missing days, all in interior points of the luteal interval.

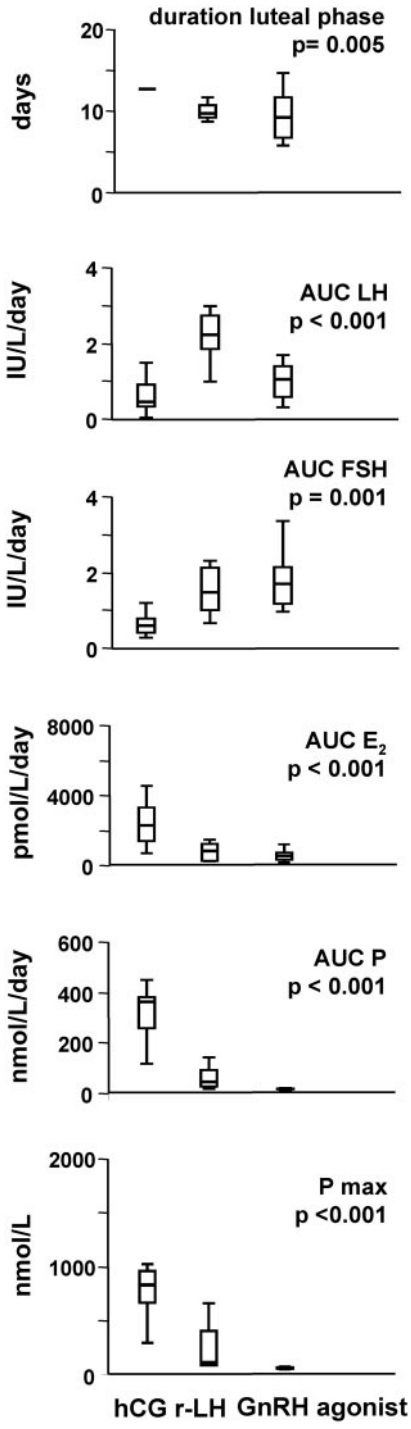

FIG. 1. Box (median values and 25th and 75th percentiles) and whisker (P_5 and P_{95}) plots representing the differences in the nonsupplemented luteal phase after induction of final oocyte maturation with either r-hCG, r-LH, or GnRH agonist in the duration of the luteal phase (calculated as interval between day of r-hCG, r-LH, or GnRH agonist and onset of menstruation in nonpregnant patients), and AUC/d of LH, FSH, E_2, and P. To determine differences in luteal hormone profiles, the AUC estimated from d 4 after randomization (to exclude the influence of the LH surge) was divided by the number of days, because the duration of the luteal phase showed a large variation. This value indicates a mean level of LH, E_2, and P on each day of the luteal phase (in nonpregnant patients). Differences in maximum P (P max) levels among the three groups are shown.

Endocrine profiles of all patients, including the pregnant patients per randomized group, are depicted in Fig. 2. Because blood sampling was carried out until 16 d post randomization, some endocrine data relate to the follicular phase of the next cycle. To address this and to study the endocrine characteristics associated with the length of the luteal phase, we elected to reallocate the nonpregnant patients (n = 34) to three groups according to luteal phase duration. Before further analysis, the patients were arbitrarily divided into groups based on luteal phase lengths of no more than 9 d, 10–12 d, and at least 13 d, resulting in 10, 14, and 10, patients, respectively.

The Kruskal Wallis test was used to test the different parameters for significance between the three groups. For the number of patients achieving embryo transfer, pregnancy, and ongoing pregnancy, 2-by-3 cross-tables were produced and analyzed by Fisher's exact test. *Post hoc* comparisons between two groups were not performed because no specific hypotheses existed before starting the study. ANOVA on log-transformed data was used to test whether differences among the three randomized groups were dissimilar in the two centers. Associations between continuous parameters were calculated by means of Spearman's rank correlation. P values < 0.05 were considered to indicate significant differences.

Results

Before recruitment to the study was completed, it became apparent that the length of the luteal phase was greatly diminished in all three study groups (Fig. 1) and that pregnancy rates were unacceptably low (Table 1). The decision was therefore made to cancel this study after 40 patients were included. At this point, only two pregnancies in the r-hCG and one pregnancy in the GnRH agonist group had been obtained. This pregnancy rate of 7.5% per started cycle was significantly lower than the 22% previously reported by our group (28). One patient, randomized to the r-hCG group, was excluded from further analyses because of premature ovulation.

The overall median age of patients participating in this study was 33.6 (range, 27.4–38.5) yr, median cycle length was 28 (range, 24–32 d) d, and median duration of infertility was 26 (range, 4–105) months. Median early follicular phase FSH levels were 6.5 (range, 2.6–16.3) IU/liter. Median late follicular phase levels (day of randomization) were: E_2, 4,558 (range, 1,137–34,137) pM; LH, 1.7 (range, 0.5–10.8) IU/liter; and P, 3.3 (range, 1.5–14.6) nM. These prerandomization parameters did not differ among groups (data not shown).

Follicular phase and luteal phase characteristics of the treatment cycle, comparing the three different oocyte maturation strategies, are shown in Table 1 and Fig. 1. Correcting for center did not change any of these findings. The group by center interaction in ANOVA (which tests for a center effect) was never significant except for P AUC ($P = 0.04$). Prerandomization parameters, including duration of the follicular phase, days of GnRH antagonist administration, and number of follicles of at least 11 mm were not different among groups (Table 1). Clinical outcome parameters, including number of oocytes retrieved, number of embryo transfers, and pregnancy rates, were not significantly different among groups (Table 1). The percentage of mature metaphase II (M II) oocytes could only be assessed in the patients undergoing ICSI. In the r-hCG group, eight patients underwent ICSI, and 85% of the oocytes were M II. For the r-LH group, five patients underwent ICSI, and 80% of the oocytes were M II. Finally, in the GnRH agonist group, eight patients underwent ICSI, and 83% of the oocytes were M II ($P = 0.9$). Hence,

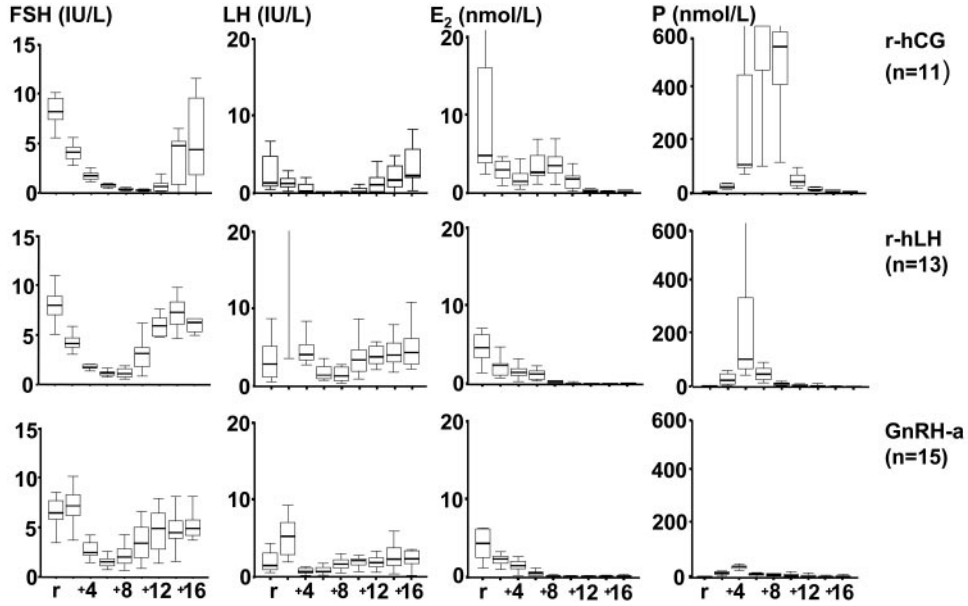

FIG. 2. Box (median values and 25th and 75th percentiles) and whisker (P_5 and P_{95}) plots representing FSH, LH, E_2, and P serum concentrations in all 39 subjects (with or without pregnancy) in the nonsupplemented luteal phase after induction of final oocyte maturation with either r-hCG, r-LH, or GnRH agonist. On the x-axis, the days of blood sampling are given. r, Day of randomization; +4, 4 d after randomization, *i.e.* day of oocyte pick up; +8, 8 d after randomization; and likewise for +12 and +16.

TABLE 1. Follicular and luteal phase characteristics (median and ranges) of 39 subjects undergoing ovarian stimulation for IVF using r-hFSH/GnRH antagonist, randomized for three different strategies for the induction of final oocyte maturation

	r-hCG (n = 11)	r-LH (n = 13)	GnRH agonist (n = 15)	*P* value[a]
Duration follicular phase (d)	11 (9–14)	12 (10–14)	12 (9–16)	0.9
No. days GnRH antagonist	4 (3–8)	4 (3–6)	4 (2–7)	1.0
No. follicles ≥ 11 mm	7 (5–16)	8 (2–18)	9 (3–13)	0.8
No. oocytes retrieved	7 (3–23)	7 (1–26)	10 (1–17)	0.9
No. patients achieving embryo transfer[b]	9	11	14	0.4
Pregnancy[b]	2 (18%)	1 (8%)	2 (13%)	0.8
Ongoing pregnancy[b]	2 (18%)	0 (0%)	1 (7%)	0.3
$LH_{(day\ of\ oocyte\ retrieval)}$ (IU/liter)	1.3 (0.3–2.9)	50.6 (3.7–54.1)	5.5 (2.0–9.6)	<0.001
Day of $P_{maximum}$	6 (6–8)	4 (4–6)	4 (4–6)	<0.001
Day of decrease of P	8 (6–8)	4 (4–8)	4 (4–8)	<0.001

[a] Parameters were tested for significance using Kruskal Wallis test.
[b] Calculated per randomized group and tested for significance using a two-tailed Fisher's exact test.

it seems that all three methods resulted in adequate final oocyte maturation.

Endocrine profiles of all patients (including the pregnant patients) per randomized group are depicted in Fig. 2. Luteal phase patterns of both gonadotropin and steroid levels were significantly different among groups, as reflected by large differences in the AUC/d for FSH, LH, E_2, and P (Fig. 1).

The luteal phase hCG levels are depicted in Fig. 3. In patients who received r-hCG to trigger final oocyte maturation, hCG levels remained detectable until 10 d post randomization, as shown before (3). In pregnant patients, hCG levels began to increase 12 d post randomization, corresponding, on average, with 4 d after implantation. The two pregnant patients treated with r-hCG showed a decline of hCG to 6 and 3 IU/liter on d 10 post randomization. In five patients, a pregnancy was confirmed by a positive pregnancy test between 15–20 d post randomization. Three patients presented with an ongoing pregnancy (*i.e.* two singleton pregnancies and one twin pregnancy). One patient suffered from an ectopic pregnancy and one from an early miscarriage. In five other patients, hCG levels showed a slight rise, up to 6 IU/liter at d 12 to 14 post randomization, despite menses. In these patients, embryo implantation may have occurred without progressing to a clinical pregnancy.

Possible correlations between late follicular phase E_2 concentrations or luteal phase AUC LH, along with correlations between AUC LH and AUC P and duration of the luteal phase for women randomized for either r-LH or GnRH agonist, are shown in Fig. 4.

Discussion

This study demonstrates, for the first time, that the nonsupplemented luteal phase is abnormal after ovarian stimulation and GnRH antagonist cotreatment for IVF. This finding was associated with such a low pregnancy rate that it was deemed unethical to complete the study as originally designed. Given that all patients have been cotreated with GnRH antagonists, the degree of abnormality of the luteal phase was striking.

Ovarian stimulation protocols for IVF normally include the coadministration of GnRH agonists to prevent premature luteinization. The resulting down-regulation of the pituitary also leads to highly suppressed luteal phase LH levels, because pituitary recovery after cessation of GnRH agonist takes 2–3 wk (3, 5, 12). In contrast, the recovery of pituitary LH release is almost immediate after the cessation of GnRH antagonist administration (13–16). This was reported for

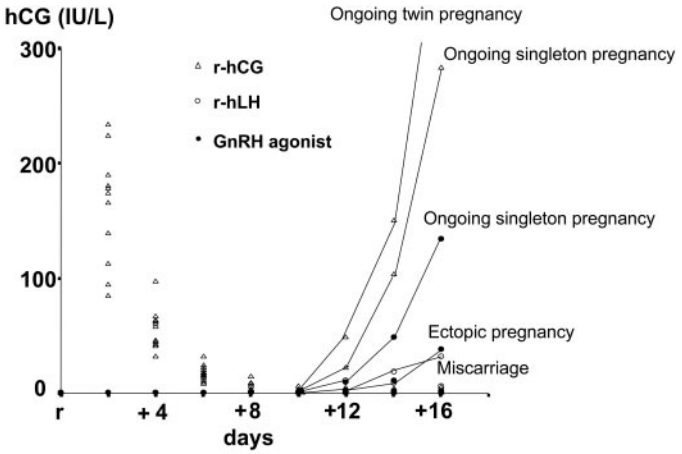

FIG. 3. hCG levels related to the time after induction of final oocyte maturation with either r-hCG, r-LH, or GnRH agonist. On the x-axis, the days of blood sampling are given.

both ganirelix and for cetrorelix available on the market. For antide, a GnRH antagonist used in the current study, rapid recovery after cessation was also shown after a week of daily injections in males (14). As-yet-unpublished data also show the clinical feasibility of the use of antide in IVF patients. A dose-dependent LH suppression was observed, involving daily administration of antide doses ranging from 0.25–2 mg (Lambalk, personal communication). The results of the current study demonstrate, for the first time, that a rapid recovery of LH release does not occur after ovarian stimulation for IVF and GnRH antagonist cotreatment. Alternative explanations for the abnormal luteal phase under these conditions are therefore warranted.

In the normoovulatory cycle, the midcycle LH surge induces final oocyte maturation, luteinization of granulosa and theca cells, and rupture of the Graafian follicle (29, 30). LH also acts as a luteotropic hormone, because it promotes the growth and the maintenance of the corpus luteum (30–32). Indeed, animal and human studies have confirmed that withdrawal of LH (by either cessation of exogenous support in the hypogonadotropic hypogonadism model or by administering a GnRH analog) induces the initiation of luteolysis (33, 34), although the corpus luteum can survive the lack of support for a limited number of days (35). In stimulated cycles, luteal endocrine characteristics are dramatically altered (1, 3, 6, 11), leading to premature luteolysis in nonsupplemented patients. As in the natural cycle, the formation and maintenance of corpora lutea in stimulated cycles are also dependent on sufficient support by endogenous LH. When a large bolus dose of hCG is used to induce final oocyte maturation in the late follicular phase, it also acts as a luteotropic agent, and the corpora lutea are supported for 7–10 d (3). After this period, clearance of the exogenous hCG from the circulation is complete, and the maintenance of the nonsupported corpora lutea becomes dependent on endogenous LH production. Should LH levels be suppressed in this phase, early luteolysis will occur.

Previously, our group studied LH surge characteristics using different late follicular phase interventions in IVF patients using luteal phase supplementation (23). In the current

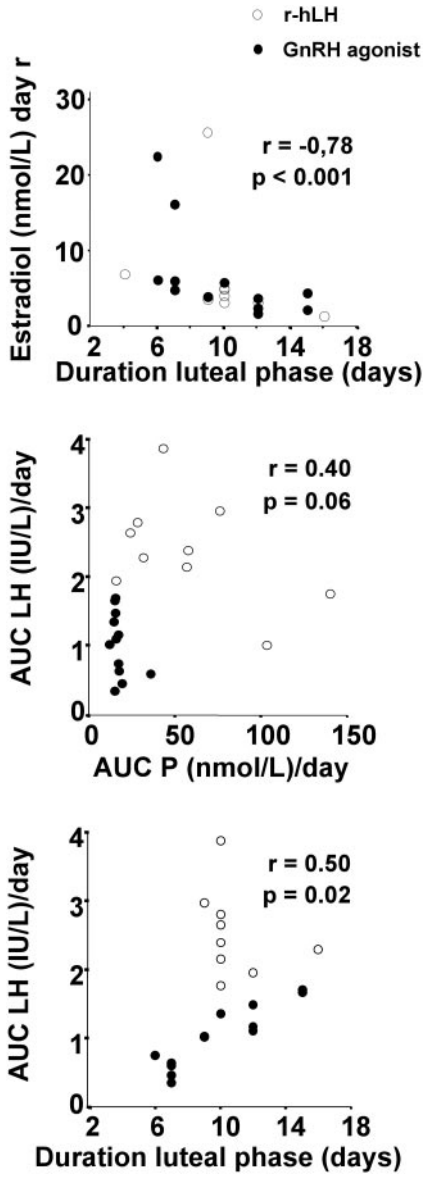

FIG. 4. Scatter plots representing the correlation between E_2 levels on the day of randomization (r) *vs.* the duration of the luteal phase (*upper panel*); between the AUC/d of LH *vs.* the AUC/d of P [both estimated from d 4 after randomization (to exclude the influence of the LH surge) divided by number of days] (*middle panel*); and between AUC/d of LH *vs.* the duration of the luteal phase (*lower panel*). All three scatterplots represent data after induction of final oocyte maturation with either r-LH or GnRH (not r-hCG).

study, the nonsupplemented luteal phase LH profile and corpus luteum function were closely monitored. The LH surge clearly differed between the study groups, with the highest levels assessed on the day of oocyte retrieval occurring in women receiving r-LH. In contrast, LH levels in those women receiving GnRH agonist were low, in agreement with a relatively short duration of the induced endogenous LH surge, as shown previously (23). In both the r-LH group and those receiving GnRH agonist, the duration of the LH surge was relatively short, with median LH levels less than 5 IU/liter and less than 2 IU/liter, respectively, being observed 4 d post randomization (Fig. 2). Because LH was assessed every

other day, the precise characteristics of the LH surges could not be assessed. The differences in observed LH levels did not seem to have any impact on the induction of final oocyte maturation itself, because the percentage of MII oocytes seemed to be normal in all three groups (36). However, in those patients receiving r-LH or GnRH agonist, early luteal phase LH levels on d 2 and 4 post randomization were positively correlated to P production, expressed as AUC/d ($r = 0.62$, $P = 0.002$ for d 2; and $r = 0.69$, $P < 0.001$ for d 4, respectively). Therefore, the lower the LH levels in the early luteal phase, the lower the P production throughout the luteal phase. This finding is consistent with earlier primate data that showed that LH surges with a duration less than 48 h are insufficient to support, or even induce, the corpus luteum (37).

After the end of the LH surge, continued luteal LH support is necessary to prevent early luteolysis and, subsequently, shortening of the luteal phase. In contrast to the previously described rapid recovery in pituitary function after cessation of GnRH antagonist (15), luteal phase LH levels in all three groups were found to be impaired. Midluteal LH levels represented by the AUC/d for LH (arbitrarily assessed from d 4 post randomization until menses) was also positively correlated with AUC/d for P and with duration of luteal phase (Fig. 4). The longest median duration of the luteal phase was observed in the r-hCG group ($P < 0.001$), again suggesting extended corpus luteum support by hCG (Fig. 1).

Both luteal E_2 and P concentrations were significantly higher in the r-hCG group, compared with both other groups, which may explain low LH levels through negative steroid feedback activity. The suppressive effect of E_2 on pituitary LH release in the luteal phase has been previously demonstrated (6). The strong negative correlation in those patients not receiving r-hCG between E_2 levels on the day of randomization (associated with follicle number) and the duration of the luteal phase (Fig. 4) provides further indirect evidence that steroid levels determine luteal phase characteristics. Those patients with a luteal phase length less than 9 d presented with higher median E_2 levels (6238 pM), compared with patients with a longer luteal phase length (3847 and 2263 pM in the groups with a luteal phase length of 10–12 d and ≥13 d, respectively). These observations imply that mechanisms other than follicular phase GnRH analog cotreatment are involved in the occurrence of suppressed luteal phase gonadotropins.

In conclusion, our data demonstrate that the luteal phase is insufficient after ovarian stimulation for IVF in combination with daily GnRH antagonist. This is the case whether r-hCG, r-LH, or GnRH agonist is used to trigger final oocyte maturation. The present study suggests that the insufficient luteal phase is principally related to supraphysiological steroid levels in the late follicular and early luteal phase (which are both related to the number of developing follicles and subsequent corpora lutea). Luteal support should therefore be provided after ovarian stimulation combined with GnRH antagonist.

Acknowledgments

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References

1. **Smitz J, Ron-El R, Tarlatzis BC** 1992 The use of gonadotrophin releasing hormone agonists for *in vitro* fertilization and other assisted procreation techniques: experience from three centres. Hum Reprod 7(Suppl 1):49–66
2. **Soliman S, Daya S, Collins J, Hughes EG** 1994 The role of luteal phase support in infertility treatment: a meta-analysis of randomized trials. Fertil Steril 61: 1068–1076
3. **Beckers NG, Laven JS, Eijkemans MJ, Fauser BC** 2000 Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for *in vitro* fertilization. Hum Reprod 15:43–49
4. **Smitz J, Erard P, Camus M, Devroey P, Tournaye H, Wisanto A, Van Steirteghem AC** 1992 Pituitary gonadotrophin secretory capacity during the luteal phase in superovulation using GnRH-agonists and HMG in a desensitization or flare-up protocol. Hum Reprod 7:1225–1229
5. **Donderwinkel PF, Schoot DC, Pache TD, de Jong FH, Hop WC, Fauser BC** 1993 Luteal function following ovulation induction in polycystic ovary syndrome patients using exogenous gonadotrophins in combination with a gonadotrophin-releasing hormone agonist. Hum Reprod 8:2027–2032
6. **Messinis IE, Templeton AA** 1987 Disparate effects of endogenous and exogenous oestradiol on luteal phase function in women. J Reprod Fertil 79:549–554
7. **Hohmann FP, Laven JS, de Jong FH, Eijkemans MJ, Fauser BC** 2001 Low-dose exogenous FSH initiated during the early, mid or late follicular phase can induce multiple dominant follicle development. Hum Reprod 16:846–854
8. **Garcia J, Jones GS, Acosta AA, Wright Jr GL** 1981 Corpus luteum function after follicle aspiration for oocyte retrieval. Fertil Steril 36:565–572
9. **Vargyas J, Kletzky O, Marrs RP** 1986 The effect of laparoscopic follicular aspiration on ovarian steroidogenesis during the early preimplantation period. Fertil Steril 45:221–225
10. **Smitz J, Devroey P, Van Steirteghem AC** 1990 Endocrinology in luteal phase and implantation. Br Med Bull 46:709–719
11. **Fauser BC, Devroey P** 2003 Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. Trends Endocrinol Metab 14:236–242
12. **Sungurtekin U, Jansen RP** 1995 Profound luteinizing hormone suppression after stopping the gonadotropin-releasing hormone-agonist leuprolide acetate. Fertil Steril 63:663–665
13. **Ditkoff EC, Cassidenti DL, Paulson RJ, Sauer MV, Paul WL, Rivier J, Yen SS, Lobo RA** 1991 The gonadotropin-releasing hormone antagonist (Nal-Glu) acutely blocks the luteinizing hormone surge but allows for resumption of folliculogenesis in normal women. Am J Obstet Gynecol 165:1811–1817
14. **Fattinger KE, Verotta D, Porchet HC, Munafo A, Le Cotonnec JY, Sheiner LB** 1996 Modeling a bivariate control system: LH and testosterone response to the GnRH antagonist antide. Am J Physiol 271:E775–E787
15. **Oberye JJ, Mannaerts BM, Huisman JA, Timmer CJ** 1999 Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Antagon/Orgalutran). Part II. Dose-proportionality and gonadotropin suppression after multiple doses of ganirelix in healthy female volunteers. Fertil Steril 72:1006–1012
16. **Elter K, Nelson LR** 2001 Use of third generation gonadotropin-releasing hormone antagonists in *in vitro* fertilization-embryo transfer: a review. Obstet Gynecol Surv 56:576–588
17. **Ragni G, Vegetti W, Baroni E, Colombo M, Arnoldi M, Lombroso G, Crosignani PG** 2001 Comparison of luteal phase profile in gonadotrophin stimulated cycles with or without a gonadotrophin-releasing hormone antagonist. Hum Reprod 16:2258–2262
18. **Albano C, Smitz J, Tournaye H, Riethmuller-Winzen H, Van Steirteghem A, Devroey P** 1999 Luteal phase and clinical outcome after human menopausal gonadotrophin/gonadotrophin releasing hormone antagonist treatment for ovarian stimulation in *in vitro* fertilization/intracytoplasmic sperm injection cycles. Hum Reprod 14:1426–1430
19. **de Jong D, Macklon NS, Fauser BC** 2000 A pilot study involving minimal ovarian stimulation for *in vitro* fertilization: extending the "follicle-stimulating hormone window" combined with the gonadotropin-releasing hormone antagonist cetrorelix. Fertil Steril 73:1051–1054
20. **Tavaniotou A, Albano C, Smitz J, Devroey P** 2002 Effect of clomiphene citrate on follicular and luteal phase luteinizing hormone concentrations in *in vitro* fertilization cycles stimulated with gonadotropins and gonadotropin-releasing hormone antagonist. Fertil Steril 77:733–737
21. **Gonen Y, Balakier H, Powell W, Casper RF** 1990 Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for *in vitro* fertilization. J Clin Endocrinol Metab 71:918–922
22. **Emperaire JC, Ruffie A** 1991 Triggering ovulation with endogenous lutein-

izing hormone may prevent the ovarian hyperstimulation syndrome. Hum Reprod 6:506–510
23. **Fauser BC, de Jong D, Olivennes F, Wrambsi H, Tay C, Itskovitz-Eldor J, van Hooren HG** 2002 Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after co-treatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for *in vitro* fertilization. J Clin Endocrinol Metab 87:709–715
24. **The European Recombinant LH Study Group** 2001 Human recombinant luteinizing hormone is as effective as, but safer than, urinary human chorionic gonadotropin in inducing final follicular maturation and ovulation in *in vitro* fertilization procedures: results of a multicenter double-blind study. J Clin Endocrinol Metab 86:2607–2618
25. **Cahill DJ, Wardle PG, Harlow CR, Hunt LP, Hull MG** 2000 Expected contribution to serum oestradiol from individual ovarian follicles in unstimulated cycles. Hum Reprod 15:1909–1912
26. **The European Recombinant Human Chorionic Gonadotrophin Study Group** 2000 Induction of final follicular maturation and early luteinization in women undergoing ovulation induction for assisted reproduction treatment—recombinant HCG versus urinary HCG. Hum Reprod 15:1446–1451
27. **Chang P, Kenley S, Burns T, Denton G, Currie K, DeVane G, O'Dea L** 2001 Recombinant human chorionic gonadotropin (rhCG) in assisted reproductive technology: results of a clinical trial comparing two doses of rhCG (Ovidrel) to urinary hCG (Profasi) for induction of final follicular maturation in *in vitro* fertilization-embryo transfer. Fertil Steril 76:67–74
28. **Hohmann FP, Macklon NS, Fauser BC** 2003 A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist co-treatment for *in vitro* fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab 88:166–173
29. **Shoham Z, Schacter M, Loumaye E, Weissman A, MacNamee M, Insler V** 1995 The luteinizing hormone surge—the final stage in ovulation induction: modern aspects of ovulation triggering. Fertil Steril 64:237–251
30. **McCracken JA, Custer EE, Lamsa JC** 1999 Luteolysis: a neuroendocrine-mediated event. Physiol Rev 79:263–323
31. **Van de Wiele RL, Bogumil J, Dyrenfurth I, Ferin M, Jewelewicz R, Warren M, Rizkallah T, Mikhail G** 1970 Mechanisms regulating the menstrual cycle in women. Recent Prog Horm Res 26:63–103
32. **Filicori M, Butler JP, Crowley Jr WF** 1984 Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. J Clin Invest 73:1638–1647
33. **Collins RL, Sopelak VM, Williams RF, Hodgen GD** 1986 Prevention of gonadotropin-releasing hormone antagonist induced luteal regression by concurrent exogenous pulsatile gonadotropin administration in monkeys. Fertil Steril 46:945–953
34. **Duffy DM, Stewart DR, Stouffer RL** 1999 Titrating luteinizing hormone replacement to sustain the structure and function of the corpus luteum after gonadotropin-releasing hormone antagonist treatment in rhesus monkeys. J Clin Endocrinol Metab 84:342–349
35. **Weissman A, Loumaye E, Shoham Z** 1996 Recovery of corpus luteum function after prolonged deprivation from gonadotrophin stimulation. Hum Reprod 11:943–949
36. **Junca AM, Mandelbaum J, Belaisch-Allart J, Salat-Baroux J, Plachot M, Antoine JM, Mayenga JM, Delafontaine M, Cohen J** 1995 Oocyte maturity and quality: value of intracytoplasmic sperm injection. Fertility of microinjected oocytes after *in vitro* maturation. Contracept Fertil Sex 23:463–465
37. **Chandrasekher YA, Hutchison JS, Zelinski-Wooten MB, Hess DL, Wolf DP, Stouffer RL** 1994 Initiation of periovulatory events in primate follicles using recombinant and native human luteinizing hormone to mimic the midcycle gonadotropin surge. J Clin Endocrinol Metab 79:298–306